UDC 547.586.2 : 576.851.132.095.3 : 577.152

142. Etsuko Toyoura : Studies on Acylase Activity and Microorganisms. XIII.*2

Optical Resolution of *p*-Methoxyphenylalanine and 3,4-Methylenedioxyphenylalanine by Metabolism of Soil Bacteria on Benzoyl Derivatives of DL-Amino Acids.

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p-Methoxyphenylalanine has not been isolated from proteins, but it was obtained as a hydrolysis product of an antibiotic, puromycin.¹⁾ 3,4-Methylenedioxyphenylalanine has not occurred in nature yet.

In the earlier papers²⁻⁴⁾ it was reported that two strains (KT-231 and KT-230) of soil bacteria metabolized benzoyl derivatives of ϵ -N-benzoyl-DL-lysine, DL-methionine, DL-phenylalanine, DL-leucine, DL-glutamic, and DL-aspartic acids to produce L-amino acids and the corresponding benzoyl-D-amino acids.

The present work is a direct continuation of that previously reported and describes the optical resolution of *p*-methoxyphenylalanine (II) and 3,4-methylenedioxyphenylalanine (V). KT-231 metabolized benzoyl derivatives of *p*-methoxy-DL-phenylalanine and 3,4-methylenedioxy-DL-phenylalanine to yield *p*-methoxy-L-phenylalanine (III), $[\alpha]_D^{10} -8^\circ$, benzoyl-*p*-methoxy-D-phenylalanine (IV), m.p. 135° , $[\alpha]_D^{10} -7.3^\circ$, 3,4-methylenedioxy-L-phenylalanine (VI), $[\alpha]_D^{10} -15.0^\circ$, and benzoyl-3,4-methylenedioxy-D-phenylalanine (VII), m.p. 126° , $[\alpha]_D^{15} +11.0^\circ$.

The metabolic activity of KT-231 and KT-230 is shown in Table I.

TABLE I. The Metabolic Activity of Soil Bacteria KT-230 and KT-231

	KT-230	KT-231
Benzoic Acid (I)	+	+
Benzoyl- <i>p</i> -methoxy-DL-phenylalanine (II)	+	+
Benzoyl- <i>p</i> -methoxy-DL-phenylalanine (II) (without NH ₄ Cl)	-	-
Benzoyl- <i>p</i> -methoxy-D-phenylalanine (IV)	-	-
<i>p</i> -Methoxy-DL-phenylalanine	-	-
Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V)	+	+
Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V) (without NH ₄ Cl)	-	-
Benzoyl-3,4-methylenedioxy-D-phenylalanine (VII)	-	-
3,4-methylenedioxy-DL-phenylalanine	-	-

+ There were luxuriant growths of bacteria within 4 days at 25° on a culture medium with the particular organic compound as the source of carbon. This cultivation experiment was repeated 3 times in succession.

- Almost no visible growth of bacteria observed at 25° in 4 days.

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*2 This constitutes a part of a series entitled "Studies on Acylase Activity and Microorganisms" by Y. Kameda. Part XII: This Bulletin, 7, 785(1959).

1) C. W. Walker, *et al.*: J. Am. Chem. Soc., **75**, 2025(1953).2) Y. Kameda, E. Toyoura, K. Matsui, Y. Kimura, S. Kitagawa: Nature, **182**, 453(1958).

3) Y. Kameda, E. Toyoura, K. Matsui: This Bulletin, 7, 702(1959).

4) E. Toyoura: *Ibid.*, 7, 785(1959).

Grateful acknowledgement is offered to Prof. Y. Kameda for his interest and encouragement throughout this work. Thanks are also due to Takeda Pharmaceutical Industries, Ltd. for financial help and to Mr. Y. Itatani for microanalyses.

Experimental

Synthesis of Benzoyl-*p*-methoxy-DL-phenylalanine (II) and Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V)—Benzoyl-*p*-methoxy-DL-phenylalanine, m.p. 173~174°, and benzoyl-3,4-methylenedioxyphenyl-DL-alanine, m.p. 179~181°, were prepared according to the azlactone method.

Resolution of Benzoyl-*p*-methoxy-DL-phenylalanine (II) by the Metabolism of KT-231—KT-231 was inoculated into 50 cc. of the culture medium⁴⁾ mentioned above containing 1.5 g. of benzoyl-*p*-methoxy-DL-phenylalanine and incubated at 25° for 10 days. The culture medium was heated at 80° for several min. and centrifuged to remove the cellular material. The supernatant solution was adjusted to pH 4.5 with AcOH, evaporated *in vacuo* to dryness, and the residue was treated with an excess of EtOH. After a few hr. of standing at ca. 5°, the precipitate was filtered by suction, washed with EtOH, and recrystallized from H₂O. 0.3 g. (62%) of *p*-methoxy-L-phenylalanine (III) was obtained as colorless plates, $[\alpha]_D^{10} -8.0^\circ$ (c=2, 5*N* HCl). *Anal.* Calcd. for C₁₀H₁₃O₃N: C, 61.52; H, 6.71. Found: C, 61.56; H, 6.75.

The EtOH solution combined with washings from the separation of *p*-methoxy-L-phenylalanine was evaporated *in vacuo* to dryness, the residue was taken up in the minimum amount of cold water, brought to pH 1.0 by addition of HCl, and extracted with AcOEt. The extract was evaporated *in vacuo* to dryness, the residue was washed several times with petr. ether to remove BzOH, and recrystallized from acetone-benzene to 0.35 g. (46.7%) of benzoyl-*p*-methoxy-D-phenylalanine (IV) as colorless needles, m.p. 135°; $[\alpha]_D^{10} -7.3^\circ$ (c=2, *N* NaOH). *Anal.* Calcd. for C₁₇H₁₇O₄N: C, 68.21; H, 5.73. Found: C, 68.15; H, 5.83.

Resolution of Benzoyl-3,4-methylenedioxy-DL-phenylalanine (V) by Metabolism of KT-231—KT-231 was inoculated into 50 cc. of the culture medium⁴⁾ mentioned above containing 1.5 g. of benzoyl-3,4-methylenedioxy-DL-phenylalanine and incubated at 25° for 18 days. The culture medium was treated according to the resolution procedure of benzoyl-*p*-methoxyphenylalanine and afforded 0.3 g. (61.3%) of 3,4-methylenedioxy-L-phenylalanine (VI) as colorless plates, $[\alpha]_D^{10} -15.0^\circ$ (c=2, 5*N* HCl) (*Anal.* Calcd. for C₁₀H₁₁O₄N: C, 57.41; H, 5.30. Found: C, 57.49; H, 5.32) and 0.4 g. (53.4%) of benzoyl-3,4-methylenedioxy-D-phenylalanine (VII) as colorless prism, m.p. 126°, $[\alpha]_D^{10} +11.0^\circ$ (c=2, *N* NaOH) (*Anal.* Calcd. for C₁₇H₁₅O₅N: C, 65.18; H, 4.82. Found: C, 65.08; H, 4.87).

Summary

The metabolic activities of KT-231 and KT-230 were tested on *p*-methoxyphenylalanine and 3,4-methylenedioxyphenylalanine and their benzoyl derivatives (Table I).

It was demonstrated that one strain (KT-231) of soil bacteria metabolized benzoyl derivatives of *p*-methoxy-DL-phenylalanine and 3,4-methylenedioxy-DL-phenylalanine to produce L-amino acids and the corresponding benzoyl-D-amino acids.

(Received March 30, 1959)